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Serological study of BVDV and BHV-1 infections in industrial dairy herds of Arak, Iran

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Abstract

BVDV and BHV-1 are considered as worldwide, complicated and economically significant infections associated with a range of clinical syndromes in cattle. As clinical syndromes such as pneumonia, diarrhea, abortion and reproductive losses were repeatedly reported in dairy herds in suburbs of Arak, this study was planned to determine the prevalence of antibodies to BVDV and BHV-1 in industrial dairy herds in the region. For this purpose, a total of 803 serum samples from 12 non-vaccinated herds were collected between June to October 2008 and evaluated for BVDV and BHV-1 antibodies using commercially available ELISA kits. Antibodies were detected against BVDV in all herds, but only one herd was free from BHV-1. The prevalence rate of 54.3% and 35.6% was estimated for BVDV and BHV-1, respectively. In addition, statistical analysis showed significant associated seropositivtiy to both infections. The results notably exhibited that both BHV-1 and BVDV infections are highly prevalent in the region, indicating that control measures should be implemented to reduce the prevalence rate of these infections.

Key words: BVDV, BHV-1, seroprevalence, Arak, Iran

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Introduction

Bovine viral diarrhea virus (BVDV) is a *Pestivirus* in the *Flaviviridae*, prevalent in the cattle population worldwide. According to growth in cell culture, BVDV biotypes are classified as cytopathogenic (cp) and noncytopathogenic (ncp) (Lidenbach *et al*., 2007). BVDV may be spread between susceptible animals by direct contact, vertical exposure or venereal transmission (Whitmore *et al*., 1978; Meyling and Jensen, 1988; Kirkland *et al*., 1994; Rush *et al*., 2001). Congenital defects, diarrhea, reproductive failure, mucosal disease, respiratory disease and hemorrhagic syndrome are the common clinical signs of the infection; however, the majority of animals show no clinical signs. Infection in pregnant animals may lead to resorption, abortions, mummification, stillbirths, teratogenic effect or persistent infection in calves. Persistently infected (PI) animals are the main source of BVDV and shed the virus during their lifetime (Perdrizet *et al*., 1987; Baker, 1995). Moreover, mucosal disease, the malignant feature of the disease is arisen from these animals which are frequently ill-thrift (Brownlie, 1985). As a result of immunosuppression caused by both transient and persistent infections, animals become prone to develop other infectious diseases (Potgieter, 1995).

Infectious bovine rhinotracheitis (IBR) is also a highly contagious infection with worldwide occurrence. The disease caused by bovine herpesvirus 1(BHV-1), a member of the genus *Varicellovirus* within the *Herpesviridae*. Involvement of the upper respiratory tract along with conjunctivitis is the typical clinical feature of the virus, although it can also cause vulvovaginitis, balanopostitis, encephalitis and abortion (Muylkens *et al*., 2007). The virus is mainly transmitted between susceptible animals by direct contact through nasal, ocular and genital secretions. Excretion of the infection in semen is also considered to be an important route of transmission (Kahrs *et al*., 1980; Afshar and Eaglesome, 1990). Ability to establish latent infection for lifelong is another significant feature of the virus. Conditions such as transportation may lead to reactivation and re-excretion of the virus complicating the control and eradication strategies (Kutish *et al*., 1990).

BVDV and BHV-1 infections are major economic concerns in cattle industry. Both of the infections have been reported from different parts of Iran, serologically and virologically (Mirchamsy *et al*., 1970; Kargar Moakhar *et al*., 1995, 2002; Sedighinejad, 1996; Hematzadeh *et al*., 2002; Haji Hajikolaei and Seyfiabad Shapouri, 2007; Fakur and Hemmatzadeh, 2007; Talebkhan Garoussi *et al*., 2008 ; Haji Hajikolaei *et al*., 2010). In view of considerable reports of these diseases from different parts of Iran and also related clinical syndromes observed in industrial dairy cattle in suburbs of Arak city situated in central part of the country, there was a growing need for serological surveillance of the infections. Therefore, this study was conducted to investigate the prevalence of antibodies to BVDV and BHV-1 in the region. The results of this study, hopefully, provide prerequisite information to assess economic impact of these infections and also to recommend appropriate control programs.

Materials and methods

Sampling

A total number of 803 blood samples were collected using vaccutainer tubes without anticoagulant from 12 intensive dairy cattle herds in Arak area between June to October 2008. Based on cooperation of the owners, the sampled herds included 80 % (12 out of 15) of the total dairy herds in the region; the herds were not immunized against BVDV and BHV-1, and had a history of pneumonia, diarrhea, abortion and reproductive disorders. Sampling was carried out randomly (Simple random sampling) among available female animals over 24 months of age in the herds (Table 1). The blood samples were transported on ice to

the regional veterinary research center. The samples were centrifuged at 1500 × g for 10 min. The sera were harvested and stored at -20˚C until tested.

Indirect ELISA

Serum samples were tested for antibodies against BVDV and BHV-1 using an indirect ELISA kit (Bio-X Diagnostics, Belgium) according to the manufacturer's protocols.

Statistical analysis

The relation of age with the infections was considered quantitatively as the mean of age was 5.54 ± 1.64 years. Animals were also categorized based on age in three age groups as: 2-4 years, 4-6 years, and >6 years including 190, 323 and 290 animals, respectively. In addition, the herds were classified according to their size as: class A, B and C including 50-150, 151-400 and >400 animals, respectively.

Data were analyzed using SPSS 13 software. The Mann-Whitney test was used to compare quantitative variable of age with nominal variables. Differences in proportions for categorical data were compared using the Chi- Square tests. Also the correlation of two nominal variables (BVD and BHV-1 infections) was analyzed using Phi and Cramer's V. *P*-values less than 0.05 were considered significant.

Results

The results of this study are summarized in Table 1. Remarkably, all herds were seropositive against BVDV and BHV-1 but only 1 herd was free from BHV-1. Of the total number of 803 sera which were collected and evaluated by ELISA test, 436 (54.3 %) and 286 (35.6 %) were positive against BVDV and BHV-1, respectively. The seroprevalence rate of BVDV virus ranged from 19.5 % to 100 %, while that of BHV-1 varied from 0 to 98.8 % within the herds.

Statistical analysis showed no significant correlation between these infections and age of animals by Mann-Whitney and Chi- Square tests. However, significant difference was determined between these two infections and herd size ($p<0.05$). The prevalence of BHV-1 infection increased significantly with herd size from 12.7 % in group A to 33.6 % in group B with an insignificant rise from group B (33.6 %) to C (40.3 %). However the rate of BVDV showed a different trend; while it slightly increased from group A (72.5 %) to B (80 %), a significant reduction was observed from group B (80 %) to C (44.6 %) (Table 2). Using Phi and Cramer's V correlation coefficient, a significant association for involvement of the both BVD and BHV-1 infections was indicated within the herds (Table 3).

Table 1. Prevalence of antibodies to BVDV and BHV-1 in 12 dairy herds in Arak, Iran

Herd code	Number (%) of sampled animals	Number (%) of seropositive animals for	
		BVDV	BHV-1
I	165(23.6)	115(69.7)	71(43)
II	86(17.2)	51(59.3)	85(98.8)
III	36(21.2)	25(69.4)	5(13.9)
IV	36(21.2)	30(83.3)	30(83.3)
V	68(27.2)	57(83.8)	12(17.6)
VI	90(22)	41(45.6)	56(62.2)
VII	20(25)	15(75)	1(5)
VIII	20(25%)	13(65)	2(10)
IX	15(18.3)	15(100)	0(0)
X	23(23)	15(65.2)	7(30.4)
XI	24(20)	16(66.7)	3(12.5)
XII	220 (29.7)	43(19.5)	14(6.4)
total	803(22.71)	436 (54.3)	286 (35.6)

Table 2. Association between herd size and prevalence of BVDV and BHV-1.

groups	Number of animals in each group	Number (%) of seropositive animals for BVDV	Number (%) of seropositive animals for BHV-1
A	102	74(72.5)	13(12.7)
B	140	112(80)	47(33.6)
C	561	250(44.6)	226(40.3)
Pearson' Chi-square		72.38	28.86
P-value		0.000	0.000

Table 3. Association between prevalence of antibodies to BVDV and BHV-1

Antibody status	Rates
BVDV (+) & BHV-1(+)	23.91 %
BVDV (+)	30.38 %
BHV-1 (+)	11.7 %
BVDV (-) & BHV-1(-)	34 %
Correlation coefficient	0.192
P-value	0.000

Discussion

Our results indicate that BVDV and BHV-1 are widely disseminated in dairy herds around Arak, the capital city of Markazi province in central part of Iran. However, the within-herd rate of infections were considerably different. As the sera were collected from animals over 24 months of age with no history of vaccination against BVDV and BHV-1, the seropositive animals are definitely the consequence of natural exposure to these viruses. The variation in prevalence rate of the infections among the herds can be largely attributed to herd management in all aspects, especially introducing of purchased animals into the herds.

Previous cross sectional studies have reported a broad range in prevalence of BVDV antibody in different countries. BVDV is thought to be present in most cattle-raising countries, and 60-90% of adult animals are seropositive (Baker, 1987). For example, the estimated prevalence of BVDV antibody was 36% in Apure state, Venezuela, 32% in Sweden and 70% in the Argentine Pampas (Obando *et al*., 1999; Bjorkman *et al*., 2000; Gogorza *et al*., 2005). In the USA, the prevalence of antibody to BVDV was reported to be 57% in 1755 animals distributed in 119 unvaccinated cow-calf operations (Paisley *et al*., 1996). Evaluation of beef cattle in New Zealand and Uruguay showed the prevalence of BVDV 63% and 69%, respectively (Perez *et al*., 1994; Guarino *et al*., 2008). In Iran, preceding regional and national works have reported a various values for prevalence of the infection. Mirchamsy *et al* (1970) showed the presence of antibody against BVDV in 35.67% of slaughtered cattle in some provinces. Sedighinejad (1996) reported the seroprevalence of 52.6% for BVDV in dairy herds that were shown related clinical symptoms and distributed throughout the country. Also, the prevalence of BVDV in 417 cattle (beef and dairy) slaughtered at Tehran abattoir was recorded as 51.58% (Kargar Moakhar *et al*., 1995). The seroprevalence of the virus was estimated at 10% in buffalo herd kept for sperm production in Urmia (Kargar Moakhar *et al*., 2002), and 33.9% in slaughtered buffaloes at Ahvaz abattoir (Haji Hajikolaei *et al*., 2010). Evaluation of bulk milk samples in suburbs of Mashhad revealed that 89.47% of the herds had antibody against BVDV (Talebkhan Garoussi *et al*., 2008). Serological studies determined the rate of infection as 27.7% and 28.5% in Sanandaj and Ahvaz, respectively (Fakur and Hemmatzadeh, 2007; Haji Hajikolaei and Seyfiabad Shapouri, 2007). The high proportion of seropositive animals within the herds implies the presence of PI animals which are usually seronegative

but they have been proved as a significant threat for spreading BVDV and establishing infection in susceptible animals (Houe, 1999). To implement eradication and control measures, recognition, identification and removal of PI animals from the herds are primarily recommended (Harkness, 1987). This strategy can also be considered as fundamental principle to reduce the rate of infection in our country.

There are also many reports that represent a variety of prevalence of BHV-1 in all continents. In Croatia, seroprevalence of antibodies in 4 dairy farms to BVDV and BHV-1 was evaluated as 79.2% and 85.8%, respectively, while 60.8% of cows had different reproductive disorders (Biuk-Ruan *et al*., 1999). The seroprevalence of BHV-1 was estimated at 67% and 69% in Apure state, Venezuela, and Uruguay, respectively (Obando *et al*., 1999; Guarino *et al*., 2008). In India, seroepidemiologic studies showed a maximum rate of infection about 54% using c-ELISA (Nandi *et al*., 2009). The distribution of the virus has also been estimated in different parts of Iran as follows: Of 201 buffalo slaughtered in west Azerbaijan (northwest of Iran) 22 (10%) showed antibody against BHV-1 (Ghabousi *et al*., 1998). In a seroepidemiologic study carried out on 9968 sera collected from the whole country the rate of infection was estimated as 30.57% (Kargar Moakhar, 2001). In a survey, 46.68% of the all cattle in Chahar Mahal Bakhtiary province had antibody against BHV-1(Hematzadeh *et al*., 2002), also in a buffalo herd kept for sperm production in Urmia (northwest of Iran) the seroprevalence of the virus was recorded as 2.5% (Kargar Moakhar *et al*., 2002). Other serological studies reported the prevalence of the virus as 34.2% in Urmia (Morshedi *et al*., 2002), 31.48% in Ahvaz (Haji Hajikolaei and Seyfiabad Shapouri, 2006), 37% in Karaj (Afshari *et al*., 2008), 30.39% in Kerman province (Sakhaee *et al*., 2009) and 27.68% in Shiraz (Badiei *et al*., 2010). The number of seropositive animals against BHV-1 may be a proportional representative of BHV-1 carriers as the virus remains latent for lifelong following primary infection (Hage *et al*., 1996). Due to the considerable economic impact of the infection and trading restrictions, some European countries including Denmark, Finland, Sweden, Austria, Norway and province of Bolzano, Italy, have successfully eradicated the virus and control strategies have been implemented in some other countries (Nandi *et al*., 2009). However, because of high prevalence of BHV-1 in our country, efforts should be directed towards the control strategies and reducing the incidence of the infection.

This study indicates that BVDV and BHV-1 are widespread in the region, and the prevalence of exposure for both viruses is comparable to those reported from other parts of Iran and the world. We also found that a significant correlation is for seropositivity to both infections in animals which may imply that common risk factors exist for the infections as explained by Paton *et al*., 1998 or may resulted from immunosuppressive properties of these viruses. These results along with those of previous research necessitate applying control measures to reduce the economic impact of BVDV and BHV-1 infections. To achieve this aim, providing a sufficient biosecurity level is stressed. However, further studies are still required to define the epidemiological features and the genotypes of the agents in Iran.

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مطالعه سرمی ویروسهای BVDV و BHV-1 در گله های گاو شیری اطراف شهرستان اراک

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چکیده

ویروسهای BVDV وBHV-1 گسترش جهانی داشته و عامل عفونتهای مهم و پیچیده توأم با خسارات اقتصادی قابل توجه در گاو هستند. از آنجائیکه سندرمهای درمانگاهی مرتبط با این دو ویروس نظیر: پنومونی، اسهال، سقط جنین و کاهش تولید مثل در گاوداریهای اطراف شهرستان اراک مشاهده می‌شد، این مطالعه به منظور تعیین میزان شیوع سرمی BVDV وBHV-1 در گاوداریهای صنعتی شیری این ناحیه انجام پذیرفت. بدین منظور مجموعاً ۸۰۳ عدد سرم از ۱۲ گاوداری بدون سابقه واکسیناسیون بر علیه این دو ویروس در فاصله خرداد تا مهر ماه ۱۳۸۷ جمع آوری و با استفاده از کیتهای تجاری الایزا مورد ارزیابی قرار گرفتند. نتیجه این تحقیق مشخص نمود که تمام گاوداریهای مورد آزمایش دارای تیتر سرمی بر علیه ویروس BVDV و بجز یک گاوداری بقیه دارای تیتر برعلیهBHV-1 هستند. میانگین شیوع سرمی BVDV وBHV-1 به ترتیب ۵۴/۳٪ و ۳۵/۶٪ در این گاوداریها بر آورد گردید که مبین گسترش بالای عوامل فوق در منطقه می‌باشد. همچنین ارتباط آماری معنی دار برای ابتلاء توأم دامهای مورد مطالعه به این دو ویروس مشخص گردید. این نتایج همراه با نتایج تحقیقات دیگری که در کشور انجام شده، اهمیت و ضرورت بکارگیری برنامه های پیشگیری و کنترل را هرچه بیشتر مورد تأکید قرار می‌دهند.

واژگان کلیدی: BVDV, BHV-1 ، سرو اپیدمیولوژی، اراک، ایران